INDUCTION OF ACROSOME REACTION WITH THE ISOLATED CHORION IN POLYCHAETE SPERMATOZOA

BULL. MAR. BIOL. STN. ASAMUSHI, TÔHOKU UNIV., 17(3), 159–164, 1983

INDUCTION OF ACROSOME REACTION WITH THE ISOLATED CHORION IN POLYCHAETE SPERMATOZOA[1)]

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Sperm-chorion interaction was examined in the polychaete, *Tylorrhynchus heterochaetus*. In the presence of calcium ions the spermatozoa underwent acrosome reaction on the isolated chorion and adhered to it using the acrosomal process. In calcium-free media they attached to the isolated chorion without acrosome reaction. This finding suggests that a sperm-chorion binding (sperm attachment) precedes acrosome reaction in fertilization process.

The polychaete, *Tylorrhynchus heterochaetus*, is one of organisms suitable for analyzing sperm-egg interaction in fertilization process (OSANAI 1978). The spermatozoa undergo acrosome reaction on the outer surface of the chorion. The lobular acrosomal process penetrates the outer thin layer of the chorion and adheres to the surface of the thick inner layer (SATO and OSANAI, 1981, 1982). Spermatozoa adhere to the chorion isolated from the unfertilized egg (OSANAI, 1976). This suggests that the chorion triggers acrosome reaction, and leads me to examine whether the acrosome reaction is induced by the isolated chorion.

MATERIAL AND METHOD

Gametes. Swarming worms of the brackish-water polychaete, *Tylorrhynchus heterochaetus* (QUATREFAGES) were collected in Natori, Miyagi Prefecture, and stored in cold 30% sea water. Gametes were discharged from the body cavity by cutting the body wall. Unfertilized eggs were preserved in 30% sea water and sperm in ordinary sea water (cf. OSANAI, 1978).

Physiological media. Natural sea water filtered with a paper filter (Toyo-roshi No. 2) and HERBST's artificial sea water modified by MOTOMURA (OSANAI, 1975) were used as physiological solution. Divalent cation-free sea water was prepared by substituting NaCl for $CaCl_2$ and Na_2SO_4 for $MgSO_4$. To remove bivalent cation contamination ethyleneglycol-bis-(β-aminoethylether) N,N'-teraacetic acid (EGTA) was added to Ca, Mg-free sea water.

Isolation of egg chorions. Unfertilized eggs were suspended in 30% artificial sea water (3 parts of artificial sea water plus 7 parts of distilled water) and

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then gently homogenized with a Teflon homogenizer. The homogenate was centrifuged at 2000 rpm for 3 minutes. After removing the supernatant, sedimented chorions were resuspended in fresh 30% artificial sea water and then centrifuged again. After repeating the same procedure several times a semi-transparent chorion fraction was obtained. The isolated chorions were suspended in a small amount of distilled water or 30% artificial sea water and stored in a refrigerator until use.

Bioassay. Isolated chorions and diluted sperm were mixed in experimental media and then the mixture was centrifuged with a hand centrifuge for removing free spermatozoa. After washing two or three times with the centrifuge method, spermatozoa remaining on the sedimented chorions were observed with a phase-contrast microscope.

Results

Acrosome reaction on isolated chorions

The *Tylorrhynchus* spermatozoon has a cylindrical head with a round vertex. A cap-like acrosomal vesicle is located in front of the nucleus (Sato and Osanai, 1982). The acrosomal vesicle can be clearly recognized with a phase-contrast microscope.

When sperm were added to isolated chorions in natural or artificial sea water, numerous spermatozoa adhered to the chorions. All of the adhering spermatozoa underwent acrosome reaction and connected to the chorion with the acrosomal process (Fig. 1a). This sperm-chorion connection seems to correspond with the adhesion of the acrosomal process to the inner layer of the chorion, which was

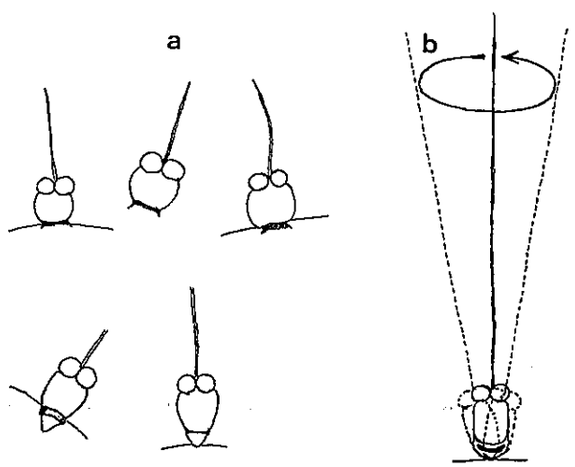

Fig. 1. Reaction of spermatozoa on isolated chorions.
a: Spermatozoa adhering to the chorion in ordinary sea water.
b: Diagram showing sperm attachment and spin movement in Ca-free sea water.

demonstrated by electron microscopy (Sato and Osanai, 1981, 1982).

Sperm-chorion attachment prior to acrosome reaction

External calcium ions are necessary for acrosome reaction in echinoderm spermatozoa (Dan, 1954; Collins and Epel, 1977). To examine whether calcium is required for the acrosome reaction of *Tylorrhynchus* spermatozoa, sperm-isolated chorion interaction under calcium-free condition was observed.

Two drops (0.1 ml) of sperm (1/10 in dilution) were suspended in 0.9 ml of Ca, Mg-free sea water and then 0.1 ml of the suspension was added to isolated chorions placed in 5 ml of artificial sea water, or Ca-free or Ca, Mg-free sea water. The chorions had been previously treated with the following procedure: the isolated chorions were suspended in Ca, Mg-free sea water containing 0.1 mM EGTA and sedimented with a hand centrifuge. The sediment was resuspended in Ca, Mg-free sea water and re-centrifuged to remove EGTA. The sedimented chorions were placed in experimental media, in which they were mixed with the sperm suspended in Ca, Mg-free sea water (Fig. 2).

When the sperm was added to the chorions in ordinary sea water, spermatozoa underwent acrosome reaction and adhered to the chorion (Fig. 3a-c). When the sperm was mixed with the chorions in Ca-free or Ca, Mg-free sea water, however,

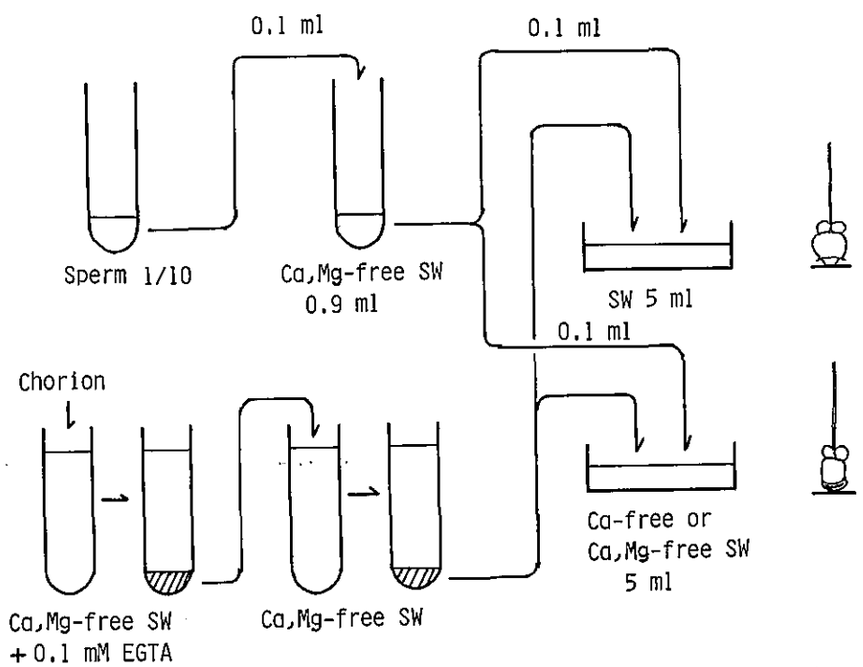

Fig. 2. Schematic representation of procedure for observing sperm-chorion interaction in Ca-free sea water.

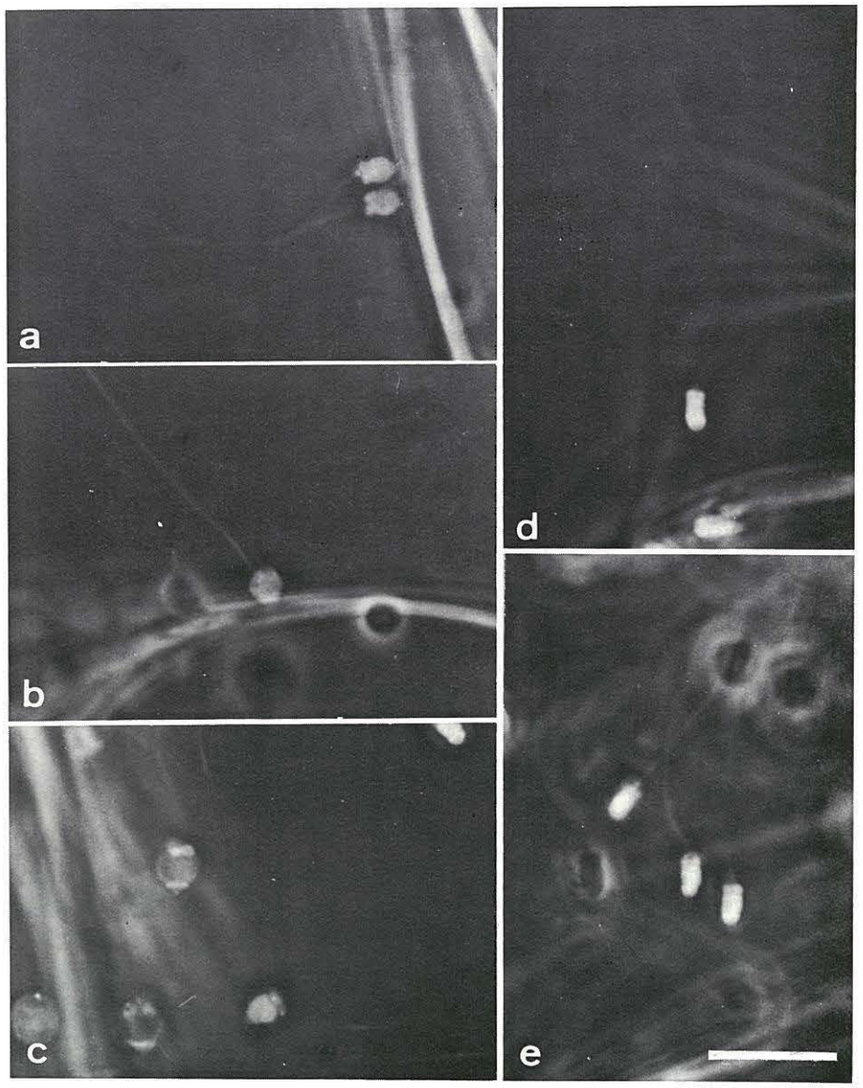

Fig. 3. Reaction of spermatozoa on isolated chorions in artificial sea water (a-c) and Ca, Mg-free sea water (d, f). × 1800. The bar indicates 10 μm.

the acrosomal vesicle remained intact. Each spermatozoon attached itself to the chorion with the tip of the head and spun the tail around the attachment point (Fig. 1b). No change in the acrosome of the attaching spermatozoa was detected with light microscopy (Fig. 3d, f). This observation suggests that the outer surface of the sperm head attaches to the chorion prior to acrosome reaction. The sperm-chorion attachment was followed by acrosome reaction in Ca-present

condition, but not in Ca-free media. Therefore, it is concluded that the presence of external calcium is required for acrosome reaction also in *Tylorrhynchus* spermatozoa.

Discussion

The results described above show that *Tylorrhynchus* spermatozoa connect with the chorion by two ways: (1) attachment prior to acrosome reaction and (2) adhesion after acrosome reaction. The former seems to correspond to the binding between the outer surface of the head tip and the outer layer of the chorion, and the latter that between the acrosomal process and the inner chorion layer (Sato and Osanai 1982). In sea urchins two ways were reported concerning sperm-vitelline layer binding. The first is the binding between sperm surface glycoproteins and vitelline layer proteins (Aketa, 1975; Aketa *et al.*, 1978). The second is the binding between vitelline layer glycoprotein and the acrosomal process by the mediation of proteinous bindin (Vacquier and Moy, 1978; Glabe and Vacquier, 1978). The mediation of an electron-dense mterial derived from the acrosomal vesicle is not found in the adhesion between the acrosomal process and the chorion in *Tylorrhynchus* (Sato and Osanai, 1981, 1982). In the process from the first attachment to the confirmed adhesion during normal *Tylorrhynchus* fertilization, an electron-dense matter is exposed by the opening of the acrosomal vesicle. The matter may mediate the binding between the acrosomal vesicle membrane and the chorion as shown in oyster spermatozoa (Brandriff *et al.*, 1978; Osanai and Kyozuka, 1982).

The progression from the sperm-chorion attachment to the acrosomal process-chorion adhesion in *Tylorrhynchus* requires external calcium ions. The acrosome

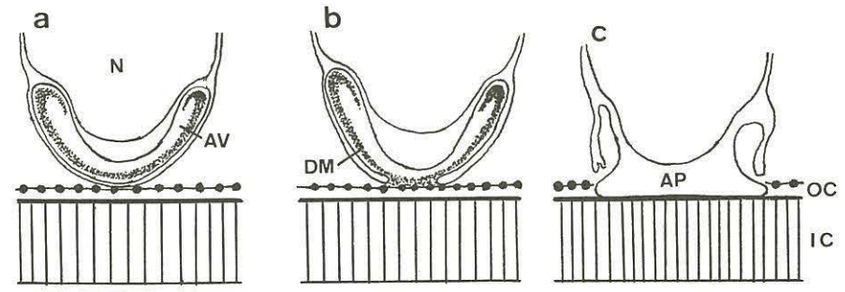

Fig. 4. Successive changes of spermatozoa on the egg chorion. Schematically drawn from electron micrographs by Sato and Osanai (1982). a: Sperm-attachment to the outer layer of the chorion. b: Intermediate step from a to c. Electron-dense matter derived from the acrosomal vesicle contacts with the outer chorion layer. c: Adhesion between the acrosomal process and the inner chorion layer.

AP, acrosomal process; AV, acrosomal vesicle; DM, electron-dense matter; IC, inner layer of chorion; N, sperm nucleus; OL, outer layer of chorion.

reaction of sea urchin spermatozoa is not induced by triggers, such as egg jelly, ionophore A23187 and alkaline, in Ca-free sea water (COLLINS and EPEL, 1977). The acrosome reaction of *Tylorrhynchus* spermatozoa may be triggered by contact with or attachment to the chorion, but can not proceed in Ca-deficient condition.

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